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When ATPases Pontin and Reptin Met Telomerase

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Pontin and reptin are conserved AAA+ ATPases identified in chromatin-remodeling complexes. In a recent issue of *Cell*, Venteicher et al. provide new insight into the function of pontin and reptin in telomerase biogenesis, which is important for cellular senescence, aging, and cancer. These unexpected findings have implications for new avenues for development of effective therapeutic drugs in human disease.

The transcription of most genes is coordinately regulated by transcriptional cofactors and chromatin-remodeling complexes (reviewed by Rosenfeld et al., 2006). Given that chromatin carries DNA and histone modifications to varying degrees and that some of these modifications are associated with transcriptional regulation, chromatin-modifying proteins that modulate epigenetic status are coming into focus. Pontin (also known as Ruvbl1, Rvb1, Tip49, Tip49a, ECP54, NMP238, TAP54α, and pontin52) and reptin (Ruvbl2, Rvb2, Tip48, Tip49b, ECP51, TAP54β, and reptin52) are AAA+ ATPases found in several chromatin-remodeling complexes believed to function in epigenetic regulation (reviewed by Gallant, 2007). From yeast to human, a variety of pontin and reptin-containing complexes have been reported (Figure 1A). Pontin and reptin are components of the INO80 and SWR1 chromatin-remodeling complexes and the Tip60 histone acetyltransferase complex, indicating that pontin and reptin function in the chromatin-remodeling process and transcriptional regulation. Further, pontin and reptin have many binding partners, including transcription factors/coregulators (i.e., c-*myc*, PROP1, NF-κB p50, TLE, Hint1, and β-catenin) and SUMO modifying enzymes (i.e., UBC9 and SENP1).

Although pontin and reptin are often found together in the same multiprotein complexes, they can act independently or exhibit opposing activities in the regulation of target genes at the mechanistic level. On the metastasis suppressor gene *KAI1* promoter, pontin is recruited together with Tip60 as a coactivator complex, whereas reptin and β-catenin function as a transcriptional corepressor complex (Kim et al., 2005). In Wnt/β-catenin signaling pathways, pontin increases the transcriptional activation of Wnt target genes, whereas reptin is a repressor of the β-catenin-TCF4 transactivation complex (Bauer et al., 2000). The pontin/reptin ratio serves to regulate heart growth via the β-catenin pathway in zebrafish embryos, suggesting functional antagonism (Rottbauer et al., 2002). In *Drosophila*, pontin has been obtained from the Brahma complex and reptin has been isolated from the PRC1 complex, and they regulate *HOX* gene transcription antagonistically (Diop et al., 2008).

Finally, pontin and reptin met an unexpected partner, telomerase (Venteicher et al., 2008). This finding makes us hold our breath to see what happens next, as the mechanism of upregulation of telomerase and the maintenance of functional telomeres in cancer cells is a highly active research area. Now, we have encountered new players in this interesting game. Telomerase plays a pivotal role in cellular senescence, aging, and cancer and has been focused on as a potential target in anticancer therapy (reviewed by Hahn, 2005; Stewart and Weinberg, 2006). Telomerase is composed of three essential components: the telomerase reverse transcriptase (TERT), the telomerase RNA component (TERC), and the TERC-binding protein dyskerin. Venteicher et al. (2008) performed affinity purification of the TERT complex from HeLa cells and identified the ATPases pontin and reptin as telomerase components. The association of pontin and reptin with TERT occurs at the endogenous level, and reptin is recruited into a TERT complex through bridging pontin.

Given these observations, what are the roles of pontin and reptin in a newly identified telomerase complex? First, pontin and reptin interact with telomerase components TERT and dyskerin and are critical for telomerase activity and for the accumulation of TERC and dyskerin. Second, pontin and reptin form a new TERT-containing complex that is highly S phase specific. The manner in which telomerase is dynamically regulated during the cell cycle has long remained unclear. Venteicher et al. (2008) proposes that S phase-specific interaction between TERT, pontin, and reptin might explain the cell cycle regulation of TERT and the assembly of telomerase in a cell cycle-dependent manner. Further, they suggest that TERT complexes are dynamic and thus that TERT protein exists in at least two different

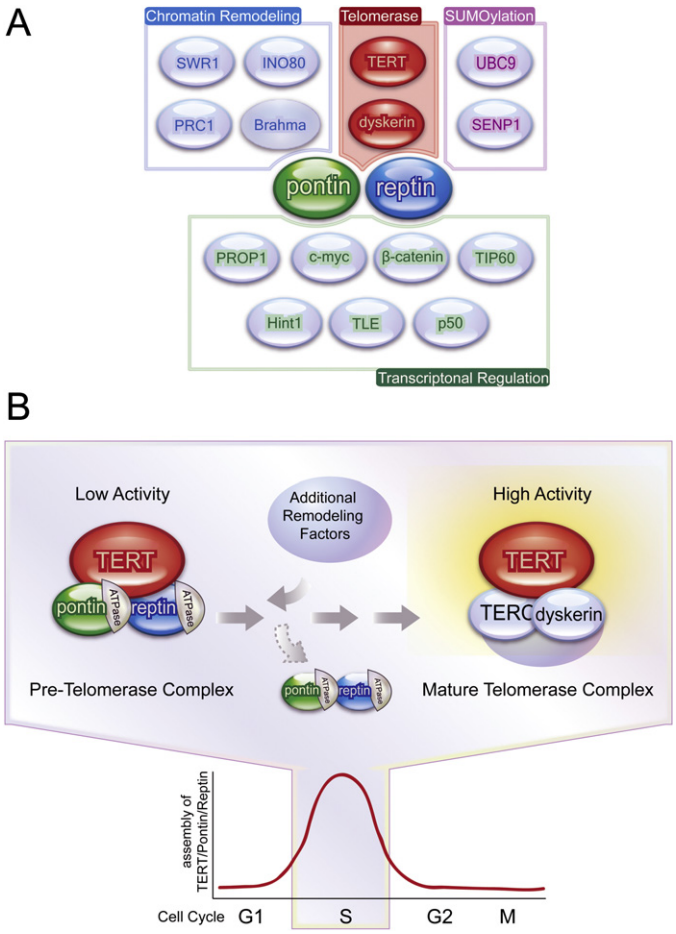

Figure 1. Schematic Representation of a Variety of Pontin and/or Reptin-Containing Complexes and Their Roles in Diverse Cellular Processes Including Telomerase Biogenesis

(A) There exists a variety of pontin and/or reptin-containing complexes involved in diverse cellular processes such as chromatin remodeling, transcription, SUMO modification, and telomerase function. Purified pontin-containing complexes possess Ubc9, whereas reptin-containing complexes contain SENP1. Pontin and reptin have been isolated from the Brahma and PRC1 complexes, respectively. Pontin and reptin have many transcription factors and coregulators as binding partners (i.e., TLE, Prop, NF-κB p50, c-*myc*, Hint1, β-catenin, and Tip60).

(B) There are multiple TERT complexes in cells. Pontin and reptin interact with TERT to assemble or remodel telomerase complexes in an S phase-specific manner. In an ordered, stepwise assembly model, the pontin/reptin/TERT complex (i.e., a pretelomerase complex) with low telomerase catalytic activity is assembled or remodeled to form the TERT/TERC/dyskerin complex (i.e., a mature telomerase complex) with high enzymatic activity.

forms (Figure 1B). One of these forms is a pontin/reptin/TERT complex (i.e., pretelomerase complex), with low telomerase catalytic activity, and the other is a TERT/TERC/dyskerin complex (i.e., mature telomerase complex), with high enzymatic activity. In an ordered, stepwise assembly model, the pontin/reptin/TERT complex is assembled or remodeled to form the TERT/TERC/dyskerin complex.

While this model adds another layer of complexity and a dynamic view of telomerase biogenesis, it also raises a host of interesting questions. When and how is the pretelomerase complex converted into a mature complex? Why does the de novo assembly of catalytically active telomerase complex occur in an S phase-specific manner? What is the function of a pretelomerase complex? Do different telomerase complexes exhibit tissue/cell specificity (i.e., normal cells versus cancer cells, embryonic stem cells versus adult stem cells)? Are there any potential mediators that modulate binding affinity of pontin and reptin with TERT? What is the upstream signal for pontin and reptin to bind and/or release TERT? While this model is attractive for now, further refinement will undoubtedly occur with the identification of additional critical components and related remodeling factors.

A potentially interesting clinical implication of these novel findings is the link between dyskerin, which is required for TERC stability, and pontin and reptin. Dyskeratosis congenital (DC) patients have abnormal skin pigmentation and nail dystrophy. These DC patients have mutations in dyskerin and thus exhibit reduced TERC levels accompanied by decreased telomerase activity and markedly shorter telomeres. It is noteworthy that the same event (i.e., loss of TERC) is associated with a depletion of pontin and reptin. This observation suggests an exciting relationship among pontin, reptin, and dyskerin and points to the potential clinical implications in DC, since both pontin and reptin appear to be involved in maintaining the expression level of dyskerin.

Collectively, this work provides new insight into the roles of pontin and reptin in telomerase biogenesis. As telomerase has been considered as a potential target for anticancer therapy, this work may provide new avenues for development of effective therapeutic drugs targeting pontin or reptin. Further, it will be of interest to address whether posttranslational modifications of pontin and reptin modulate the affinity for TERT and dyskerin. Given that SUMO modifying enzymes are obtained from pontin- and reptin-containing complexes, and SUMO modification of pontin and reptin regulates their transcriptional activity and protein-protein interaction (Kim et al., 2007), it is tempting to speculate that various posttranslational modifications of pontin and reptin might be involved in the elaborate modulation of telomerase action (i.e., epigenetic regulation of telomere length).

Considering the identified diverse functions of pontin and reptin identified thus far, it is of particular interest to speculate that formation of dynamic complexes among pontin and reptin might be tightly regulated in a promoter-, cell-, and/or signal-dependent manner and may ultimately result in different functional output. Further, potential regulatory mechanisms such as SUMOylation, ubiquitination, and methylation linked to signaling pathways might serve to coordinate and/or switch among the functions of pontin and reptin and thus affords a plausible explanation for

the existence of such diverse pontin and reptin complexes with different functions.

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Brushed Aside and Silenced

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Mammalian genomes are highly organized in the 3D space of cell nuclei, but whether this affects gene function is unclear. Three papers now show that spatial relocation of a gene directly affects expression, and surprisingly, that of its neighbors.

The relationship between nuclear structure and genome function has developed into a classic chicken-and-egg question. Over the last decade in particular, researchers have observed strong correlations between gene expression states and specific intranuclear positioning of genes. For example, on a simplistic level, active genes often occupy more internal nuclear positions, while inactive genes tend to be located toward the nuclear periphery. What hasn't been clear is whether differential localization to these and other subnuclear ''compartments'' is a cause or consequence of altered gene expression states; in other words, do genes move to specific functional compartments to get activated or silenced, or do they relocate because of the physical properties they have acquired in the process of being activated or silenced? A recent paper from Harinder Singh's group (Reddy et al., 2008) employing inducible tethering of genes to the inner nuclear membrane (INM) has made a significant crack in this conundrum. In mammalian cells the nuclear periphery is comprised of a distinct set of INM proteins, such as LBR, LAP2, and emerin (EMD), as well as an underlying network of proteins known as the nuclear lamina, which has been proposed to interact with transcriptional repressors. A genome-wide screen to identify genes associated with the nuclear lamina in *Drosophila* cells found primarily silent genes that were developmentally regulated, suggesting it may be the final resting place of previously used genes (Pickersgill et al., 2006). Reddy et al. tagged a gene construct with a series of Lac operator (*LacO*) binding sites and visualized the intranuclear position of the gene in live cells by expressing a hybrid protein. They fused the green fluorescent protein (GFP) to a domain from the LacI protein, which inducibly binds the *LacO* sequences with high specificity. Under these conditions the gene tended toward an internal nuclear position and was expressed normally. Expression of another fusion protein, this time a hybrid between GFP, LacI, and the transmembrane domain of EMD, led to relocalization of the tagged gene to the nuclear periphery and transcriptional repression (Figure 1). Interestingly, passage through mitosis was necessary for relocalization to take place. The fact that breakdown and reassembly of the nuclear membrane is required for stable peripheral relocation was elegantly demonstrated in another recent report from the Spector laboratory (Kumaran and Spector, 2008).

Importantly, the transcriptional repression at the periphery observed by Reddy et al. was not complete; some of the tethered genes still showed weak transcriptional activity. This result suggests that the periphery is not a completely silent zone, echoing earlier work in yeast revealing that silent and active compartments coexist in the periphery (Akhtar and Gasser, 2007; Taddei et al., 2006). The idea that the periphery is not universally repressive is reinforced by Kumaran and Spector, who used a similar approach to target a 4 Mb transgene array to the nuclear periphery and were able to inducibly activate it in situ.

Intriguingly, Reddy et al. also found decreased expression of endogenous genes within a few hundred kilobases of the insertion site, suggesting that a tether site could have more widespread effects.